Estimating Bacterial Populations in Raw and Simulated Maple Sap by a Modified Resazurin Test

By J. C. KISSINGER (Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, 600 E. Mermaid Lane, Philadelphia, Pa. 19118)

A modified resazurin test was used to estimate bacterial populations growing in natural and simulated maple saps. Comparable results were obtained from both substrates, with a slight decrease in dye reduction time noted when the natural sap substrate was used. Simulated sap could provide a readily available substitute for natural sap in collaborative testing.

The results of initial studies of a modified resazurin test for estimating bacterial population in maple sap were reported earlier (*JAOAC* **52**, 714–716 (1969)). The test method was re-examined and changes were made to facilitate preparation of media and to decrease the time required to perform the test. The revised method is given below.

METHOD

Apparatus and Reagents

(a) *Pipets, serological.*—To deliver 1 and 10 ml, with 1.0 ml graduations (sterilized).

(b) *Test tubes.*—150 × 16 mm, screw-top with molded plastic caps (sterilized).

(c) *Test tube racks.*

(d) *Incubator or water bath with opaque cover.*—Constant temperature, capable of holding temperature at 37.5±0.5°C.

(e) *Bottles.*—200 ml amber, glass-stoppered.

(f) *Nutrient broth.*—10-strength. Combine 30 g beef extract and 50 g peptone and dilute to 1 L with distilled water. Sterilize by filtration through 0.45 μm membrane filter or by autoclaving 15 min at 15 psig.

(g) *Resazurin dye.*—Place 200 ml distilled water in amber glass bottle and sterilize by autoclaving 15 min at 15 psig. Using sterile, dry forceps, add 1 standard resazurin dye tablet and shake to insure complete solution of dye before water cools.

Procedure

To sterile test tube, transfer 1 ml nutrient broth and 10 ml sap to be tested. Mix sap and broth by capping and inverting tube. Incubate tube 30 min at 37.5°C. After incubation, remove tube from incubator and, with sterile pipet, add 1 ml resazurin dye. Cap tube, invert to mix dye thoroughly, and incubate at 37.5°C. Examine tube for color change to pink (end point) at 30 min intervals and note time of end point.

Estimate bacterial cell population in sap from curve relating time of color development to cell count (Figs. 1 and 2).

Results and Discussion

Conventional pour plate techniques were used in making all bacterial counts made in this study. In this revised method, the substitution of membrane filtration for sterilization of the nutrient broth was preferable to autoclaving because it required less time. In addition, sap/broth mixtures made with filtered broth were lighter in color than those made with autoclaved broth and, therefore, decreased possible color interference in reading end points. The increase in incubation time of the broth/sap mixture from 15 to 30 min before the addition of the indicator dye gave a slight improvement in the time required to run the test. A positive reaction from a bacterial population of 10^7 cells/ml was obtained in 1 hr. By contrast, the same population required 1½ hr for a positive test, using the 15 min pre-incubation.

Figure 1 shows a curve in which the bacterial populations in simulated sap samples were plotted vs. the time required for reduction of resazurin dye by these microorganisms. The solid line was constructed from results obtained from 95 determinations and was fitted by the least squares method. The interrupted lines delineate the confidence limits. An analysis of variance of these data showed a significant F value ($p=0.01$).

Although the above data indicated that the method might be subjected to a collaborative

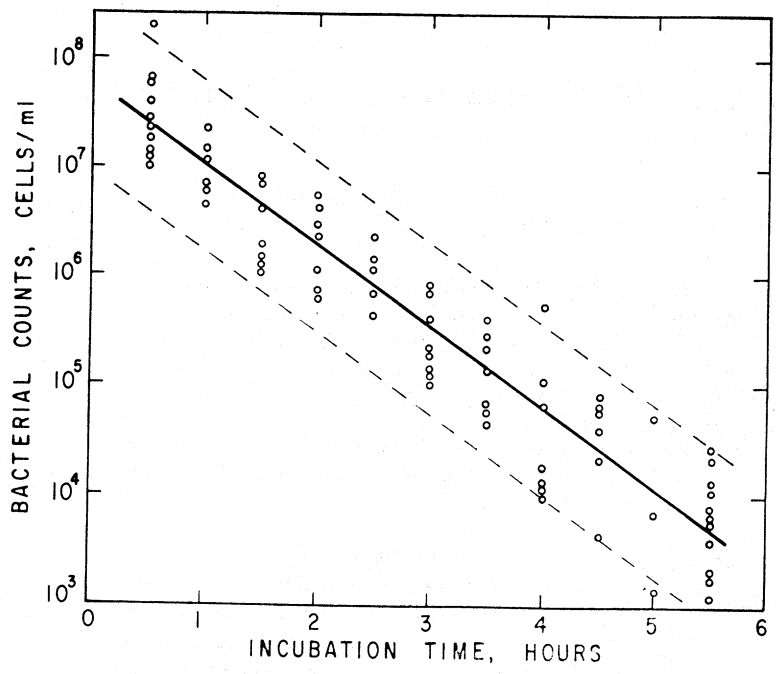

FIG. 1—Resazurin reduction time for bacterial populations in simulated maple sap.

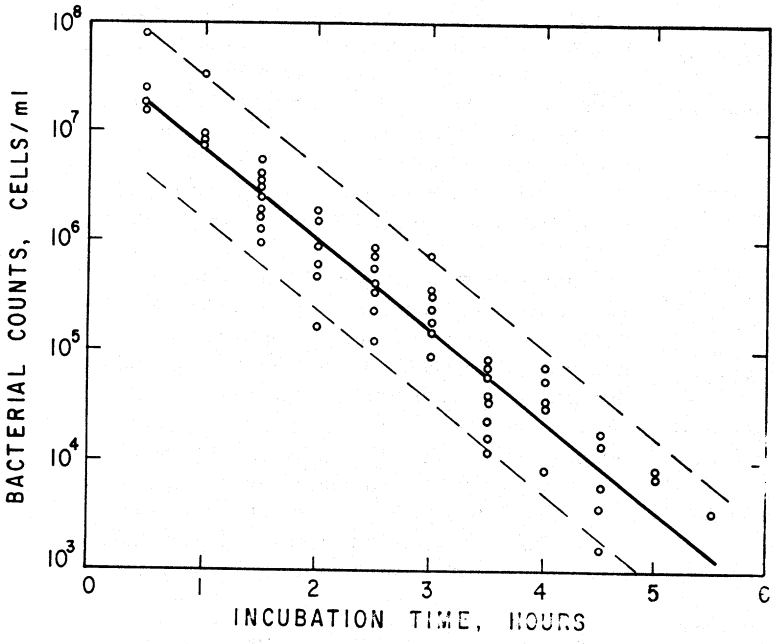

FIG. 2—Resazurin reduction time for bacterial populations in natural maple sap.

test with satisfactory results, a question remained on the validity of extrapolating data secured from microbial populations propagated in simulated sap to actual industrial operations concerned solely with raw maple sap. Simulated sap is made by diluting light amber standard density maple sirup to 2.5° Brix (average sap Brix) with distilled water. Since this does not replace the "sugar sand" (primarily calcium malate) which is precipitated from sap during the concentration process and filtered from the finished sirup, it was felt that simulated sap might be inferior to natural sap as a growth medium for sap microorganisms.

A supply of raw maple sap was secured and used to duplicate the study made with simulated sap. The results are shown in Fig. 2. This curve was similar to that of Fig. 1. In both studies, bacterial populations of 10^7 cells/ml produced positive color changes after a 1 hr incubation. An analysis of variance of these data showed a significant F value ($p = 0.01$). The slope of the line of Fig. 2 was slightly greater than that of Fig. 1, indicating that bacterial populations growing in raw sap produced a positive color change in less time than those grown in simulated sap, but the similarity between the 2 graphs indicated that the validity of this method could be tested collaboratively with the use of simulated sap rather than natural maple sap.

Recommendations

It is recommended—

(1) That the study of this method be continued.

(2) That a suitable color standard be selected to provide uniformity in end point determination.

(3) That a collaborative study be made of the method.

Acknowledgments

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